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Evaluation of Cytotoxic and Antioxidant properties and Phytochemical analysis of *Vernonia anthelmentica*.Willid. leaf extracts

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ABSTRACT
Vernonia helmentica finds a mention in Ayurvedic pharmacopoeia of India as bitter, acrid and thermogenic. It is a medicinal herb known for its anthelmintic, astringent and anti-inflammatory activity. In the present study leaf extract of the plant has been explored for its cytotoxic, antioxidant and phytochemical properties. In this study fresh sample showed the presence of terpenoids along with saponins and tannins, whereas in solvent extracts only saponins and tannins were observed. Cytotoxic activity was better in hexane extract whereas antioxidant activities was more in acetone and methanol extracts of the leaves of the plant.
KEYWORDS: *Vernonia helmentica*, cytotoxic, antioxidant and phytochemical analysis

INTRODUCTION

Medicinal plants have always provided leads for the isolation of active principles. Folk people still largely depend upon medicinal plants for their healthcare. Plant derived formulations and powders are integral part of Ayurvedic, Unani and Siddha system of medicines[1]. South Africa and Chinese herbal remedies are also well known to the world[2]. Keeping in view of the side effects of Allopathic system of medicines, medicinal plants have always remained in the forefront of health care system of people. Exploration of new medicinal plants and providing scientific basis to the plants used by tribal and folk people is very much the need of the day to strengthen the health related remedies of the mankind.

Vernonia is a large genus of herbs, shrubs and small trees found in the tropics almost all over the world. A number of species of this genus namely *Vernonia cinerea*, *Vernonia amygdalina* and *Vernonia kotschyana* are known for their anti-inflammatory activity[3], antimalarial[4], antioxidant[5]and anti-microbial activities[6].

Bioassay guided isolation from *Vernonia cinerea* has resulted in isolation of a number of new molecules of importance [7]. In the present study phytochemical profile, Cytotoxic activity and antioxidant potential of *Vernoina anthelmintica* was focused upon.

MATERIALS AND METHODS

Source of Plant Material: Leaves of the *Vernonia anthelmentica* were collected from the medicinal germplasm garden of the Regional Plant Resource Centre, Bhubaneswar. Same were authenticated with the digital herbarium of the institute.

Solvent extract preparation: After collection leaves were thoroughly washed under running tap water and then were dried in shade followed by grinding in mechanical grinder. 15 gm of powdered leaf sample of plant *Vernonia anthelmintica* was taken in a thimble & was subjected to serial extraction with different solvents like Hexane, Chloroform, Acetone, and Methanol on the basis of increasing polarity. 250ml of solvent was taken in a round bottom flask & extraction was carried out using Soxhlet apparatus,

Extracts obtained were concentrated in vacuum under pressure using Bucchi (R-200) Rotavapour. Concentrated extract was stored in screw cap vials until further use. Yield of the extracts was also recorded.

Phytochemical assays: Phytochemical assays were conducted on all the solvent extract along with the freshly prepared methanolic extract, for which leaves were directly grinded in methanol using pestle mortar and after that were filtered to get a fresh methanolic extract. Assays were conducted following the standard protocols[8].

Assessment of Cytotoxic activity: Brine shrimp mortality assay was conducted [9] for ascertaining cytotoxic potential of the solvent extracts of the leaves. Brine shrimp (*Artemia salina*) eggs were incubated for 48 hrs (3.6 gm of black salt in 200 ml distilled water) to get the desired growth of the larvae for biological evaluation. Stock solution of different extracts were prepared at a concentration of 10 microg/ml, cytotoxic assay we carried out at 5 doses 25, 50,100, 200µg/ml for each dose level, 3 replicates were used.

After 24 hrs number of live larvae was counted in all the samples. Percentage inhibition was calculated by comparing the treated samples with the controls. Standard deviation was also calculated.

Antioxidant assays of the leaf extracts:
Qualitative Analysis (TLC based antioxidant studies):

For detecting Antioxidant activity, preliminary qualitative 2, 2- Diphenyl1-picrylhydrazyl (DPPH) assay was conducted. 5µl of each sample was loaded on the TLC sheet and the chromatograms were developed in following solvent systems:

a) Ethyl acetate: methanol: water (40:5.4:4) [EMW] (polar neutral)

b) Chloroform: ethyl acetate: formic acid (5:4:1) [CEF] (Intermediate polarity/acidic)

c) Benzene: ethanol: ammonium hydroxide (90:10:1) [BEA] (Non polar/basic)

Chromatographs were sprayed with 0.2% DPPH in methanol as an indicator[10]The presence of antioxidant compounds were detected by yellow spots against a purple background on the TLC sheet sprayed with 0.2% DPPH in methanol.

$DPPH + AH \rightarrow DPPH - H + A^-$

(Purple color) (Yellow color)

Quantitative anti-oxidant Analysis:

Quantitiative analysis was done by two popular methods as follows;

DPPH free radical scavenging assay:-

For DPPH free radical scavenging assay 1mM DPPH (2, 2- Diphenyl-1- picryl hydrazyl) (Mol. Wt. 394.33) solution was prepared. 4mg DPPH was weighed and dissolved in 10ml methanol. DPPH assay was done by serial dilution method starting from concentration 1250µg, 625µg, 312.5µg, 156.25µg, 78.125µg, 39.625µg, 19.812µg, 9.9µg, 4.9 µg. There were duplicate test tube of each concentration was taken. 1ml of each sample was taken in the test tube & volume was made up to 4ml by methanol. 500µl DPPH solution was added to each test-tube and stirred thoroughly before incubated for 30min.

Ascorbic acid was used as a reference standard and dissolved in distilled water to make the stock solution with the same concentration. Control sample was prepared containing the same volume without any extracts & reference ascorbic acid. Then optical density (OD) was measured at λ = 517nm in spectrophotometer. The percentage radical scavenging activity was calculated from the following formula:

Percentage radical scavenging:

$$[DPPH] = [(Ao - A1) \div Ao] \times 100$$

Where Ao was the absorbance of control and A1 was the absorbance of sample.

FRAP Assay (Estimation of total antioxidant activity): Ferric reducing power assay was conducted following the protocol of Ahmad and Beige[11]. Absorbance was read at 593nm.

RESULTS AND DISCUSSIONS

Solvent extracts: Yield of methanol extract was maximum as shown in Table 1. to the tune of 14.4% followed by acetone and chloroform extracts at 3.2% each and least in hexane extract (2.8%). It could be clearly stated that polar compounds were more than the non- polar entities.

Phytochemical analysis: Fresh sample was found to be richest and showed the presence of terpenoids, tannin and saponins. Tannins and saponins were universally present in all the extracts. It can be concluded that terpenoids present in fresh sample was of volatile nature and extraction process resulted in its loss. Presence of tannins and saponins in the extracts indicated their medicinal potential as both the class of compounds are used in the alternative medicine[12].

Cytotoxic activity: As can be seen in Fig. 1, Hexane extract showed highest activity at a dose of 50microgram/ml, but at the higher dose its activity declined. Cytotoxic activity of the extracts was mild not much promising. A number of species of Vernonia genus, like *Vernonia amygdalina* are used as food[13] as well as in medicines. So lack of cytotoxicity of *Vernonia anthelmintica* suggests it to be safe for fodder use.

Antioxidant activity:

Qualitative TLC based assay showed a number of antioxidant bands in all the solvents. Chloroform extract was found to be richest with 7 bands, whereas hexane extract showed the least number of bands(3). Results of quantitative assays were different from the qualitative assay. Methanol extract showed the maximum potential in both DPPH as well as FRAP assay(Fig 2 & 3). Antioxidant potential of *Vernonia condensata*, *Vernonia amygdalina* and *Vernonia bluemoides* has been reported[14] and all of the above owe their antioxidant potential because of phenolics compounds like tannins and flavonoids. Thus, antioxidant potential of *Vernonia anthelmintica* could be due the tannins present in the species.

Thus, presence of tannins and saponins, mild cytotoxic activity and significant antioxidant activity of methanol extract has provided lead making it a good subject for further scientific exploratory work.

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Table 1; Yield of solvent extracts of *Vernonia anthelmentica*

Extracts	Weight of screw cap vial (A)	Weight of screw cap vial with sample (B)	Weight of extract	Percentage yield of extract
HEXANE	17.504	17.930	0.426	2.8%
CHLOROFORM	17.584	18.068	0.484	3.2%
ACETONE	17.252	17.732	0.480	3.2%
METHANOL	17.454	19.619	2.165	14.4%

Table 2: Phytochemical analysis:

Class of compounds	Fresh samples	Hexane extract	Chloroform extract	Acetone extract	Methanol extract
Tannin	+ve	+ve	+ve	+ve	+ve
Anthraquinone	-ve	-ve	-ve	-ve	-ve
Saponin	+ve	+ve	+ve	+ve	+ve
Flavonoid	-ve	-ve	-ve	-ve	-ve
Terpenoids	+ve	-ve	-ve	-ve	-ve
Alkaloid	-ve	-ve	-ve	-ve	-ve
Glycoside	-ve	-ve	-ve	-ve	-ve

Table 3: Qualitative Analysis of Antioxidant:

EXTRACTS	SOLVENT	R.F Values
HEXANE	BEA	0.21, 0.29, 0.35
	CEF	0
	EMW	0
CHLOROFORM	BEA	0.23, 0.42, 0.76
	CEF	0.57
	EMW	0.35, 0.42, 0.84

ACETONE	BEA	0.25
	CEF	0.37, 0.8
	EMW	0.32, 0.42, 0.84
METHANOL	BEA	0.23
	CEF	0.24, 0.61
	EMW	0.25

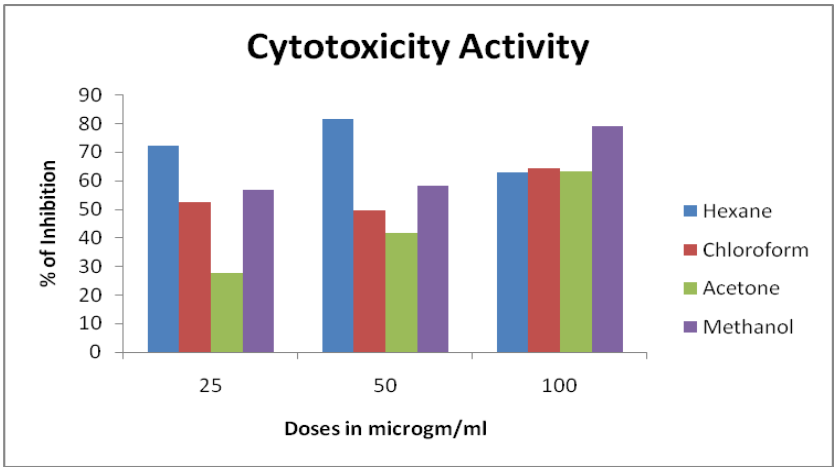

Figure 1. Brine shrimp assay of extracts of *Vernonia anthelmintic*

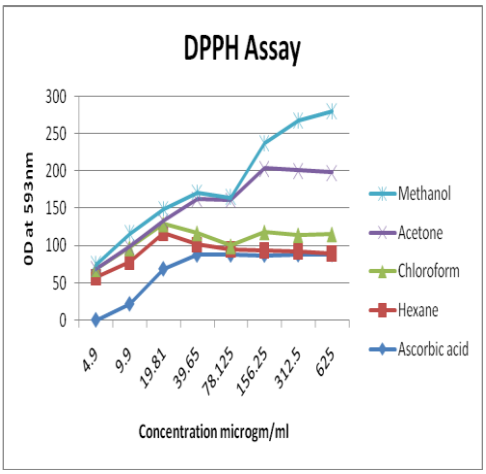

Figure 2. DPPH radical scavenging assay

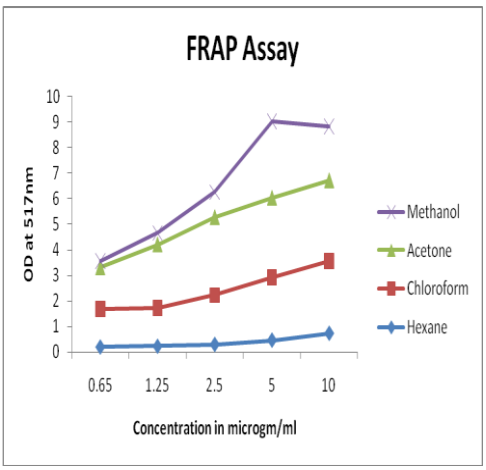

Figure 3. Ferric oxide reducing power assay